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Dietary intake of micronutrients and the risk of developing bladder cancer: results from the Belgian case–control study on bladder cancer risk

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Abstract

Objective We aimed to investigate the effect of dietary intake of micronutrients that are metabolized and excreted via the urinary tract on bladder cancer risk.

Methods A semi-quantitative 322 item food frequency questionnaire (FFQ) was used to collect dietary data from 200 bladder cancer cases and 386 control subjects participating in the Belgian case–control study on bladder cancer risk. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using unconditional logistic regression adjusting for age, sex, smoking characteristics, occupational exposures, and energy intake.

Results We observed a positive association between calcium intake and bladder cancer (OR: 1.77; 95% CI: 1.00–3.15; p -trend = 0.049) and increased odds, although not statistically significant, for highest tertile of phosphorus

intake (OR: 1.82; 95% CI: 0.95–3.49; p -trend = 0.06). We identified possible modification of the effects of both calcium and phosphorus by level of magnesium intake. Increased odds of bladder cancer were also observed for participants with highest intake of phosphorus and lowest intake of vitamin D (OR: 4.25; 95% CI: 1.44–12.55) and among older participants with the highest intakes of calcium (OR: 1.90; 95% CI: 1.08–3.36) and phosphorus (OR: 2.02; 95% CI: 1.05–3.92).

Conclusion The positive associations we observed between bladder cancer and intake of calcium and phosphorus require confirmation by other studies. The balances between inter-related micronutrients also warrant further examination.

Keywords Bladder cancer · Micronutrients · Calcium · Vitamin D

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Introduction

Bladder cancer is a major health problem particularly for aging males from Western populations [1]. Many dietary components are metabolized and excreted in the urinary tract increasing the biological plausibility that certain minerals and vitamins or their metabolites have the potential to either inhibit or promote bladder carcinogenesis [2].

A systematic review [3] reported that the essential trace mineral, selenium may have a protective effect against developing bladder cancer. Less information, however, is available on the effect of other dietary micronutrients that are present in daily urine excretion. These include electrolytes, sodium and potassium and major dietary minerals such as calcium, magnesium, and phosphorus [4].

Sodium is an essential mineral that in high doses has been shown to promote bladder carcinogenesis in rodents [4]. An all-male case–control study conducted in the US [5] consisting of 351 cases and 855 controls, also reported a positive association between sodium intake and bladder cancer incidence. Two other US studies, however, a prospective cohort study of male health professionals [6] and a case–control study [7] observed no association between sodium intake and bladder cancer. These two epidemiological studies [6, 7] also failed to detect an association with potassium. This major intracellular mineral has similar functions to sodium, although quite often in the opposite direction and according to animal studies may be involved in cellular proliferation [4, 8].

Depending on their chemical form, calcium and magnesium may increase the risk of bladder cancer through the development of precipitates/calculi, influence on urine pH and interaction with other urine components [4]. To date, however, few epidemiological studies [5–7, 9, 10] have actually examined the effect of these minerals on bladder cancer risk. Whereas a multi-centre Spanish case–control study [9] observed a positive association with bladder cancer, although this disappeared after adjusting for saturated fat intake, three US studies [5–7] and one Dutch prospective cohort study [10] reported no association with calcium intake. Inverse associations have been reported between magnesium intake and cancers at several other body sites, e.g., the colon [11], liver [12], and esophagus [13] but only two US studies [6, 7] have investigated its relationship with bladder cancer. While the case–control study [7] observed no association, the prospective study [6] reported an inverse association between magnesium and bladder cancer prior to multivariate analysis.

Phosphorus has a number of key physiological functions including its roles in bone mineralization and as an important component of nucleic acids and cellular membrane [14]. This mineral also biologically interacts with

calcium and vitamin D [15]. To our knowledge, only two epidemiological studies [6, 7] have investigated the effect of phosphorus intake in relation to bladder cancer. Following adjustment for several potential confounders, the inverse association with bladder cancer disappeared in the prospective study [6] and was borderline statistically significant (dietary phosphorus only) in the case–control study [7].

Deficiencies in some minerals and vitamins such as iron, zinc, and B group vitamins are reported to possibly increase the risk of cancer by causing damage to DNA similar to radiation exposure [16]. Although the mechanism by which copper deficiency possibly influences carcinogenesis has not been elucidated [4], it may be due to its role in the superoxide dismutase (SOD) enzymes, which help to prevent oxidative damage to the cell membranes [8]. However, the effect of dietary intake of copper on the risk of developing bladder cancer has not been investigated previously, and only few epidemiological studies have examined potential associations with dietary iron [6, 7, 9], zinc [17, 18] and vitamins B1 (thiamin) and B2 (riboflavin) [5–7, 19].

While the epidemiological evidence for any associations between micronutrients included in this study and bladder cancer is sparse and inconsistent, it is virtually non-existent for the combined effects of major inter-related micronutrients. Calcium, magnesium, phosphorus, and vitamin D not only share common food sources but are “metabolically interactive” as well [4]. Examining the effect of a single micronutrient without taking into account the influence of complementary or opposing nutrients may lead to residual confounding and incomplete risk assessment. Therefore, the aim of our study was to evaluate the effect of dietary intake of minerals and vitamins that have a biological association with bladder cancer risk, in terms of both main and potential interaction effects.

Methods

Study population

The design of the Belgian case–control study on bladder cancer risk has been described previously in detail [20]. Briefly, our study was a population-based case–control study conducted in the Belgian province of Limburg consisting of 200 bladder cancer cases and 386 healthy control subjects [20]. All cases included in the study were incident cases identified with histologically confirmed transitional cell carcinoma (TCC) of the bladder between 1999 and 2004. Cases were derived from the Limburg Cancer Registry (LIKAR) and invited to participate in the study by urologists and general practitioners. Since strict Belgian

privacy laws preclude direct access to population registers, control subjects were selected through the Belgian authority, Kruispuntbank van de Sociale Zekerheid (Crossroads Bank of Social Security). This was done via simple random sampling, stratified by municipality and social economic status among inhabitants from the province of Limburg who were 50 years of age and older, without a diagnosis of transitional cell carcinoma of the bladder. The selected individuals were invited by mail to participate in the study. The study was approved by the Ethical Review Board of the Medical School of the Katholieke Universiteit of Leuven, and all participants provided written informed consent [20].

Data collection

The IMMIDIET standardized food frequency questionnaire (FFQ) was sent by mail to all participants in the study. The IMMIDIET FFQ was developed previously for another study, “Dietary Habits Profile in European Communities with Different Risk of Myocardial Infarction: the Impact of Migration as a Model of Gene/Environment Interaction” (The IMMIDIET Project which was funded by the European Union in the 5th Framework Programme under Key Action 1: Food, Nutrition and Health, QLK1-CT-2000-00100) [21]. The IMMIDIET FFQ has been validated [22, 23] and focuses specifically on the Belgian population [21]. The semi-quantitative FFQ contains 322 food items and is linked to the combined contents of three existing food tables (the NEVO table for the Netherlands [24], the Belgian Nubel table [25], and the IPL table [26] for Francophone Belgians) supplemented with information on the composition of common recipes from the region. Participants were asked to report their usual dietary intake for the 12 months prior to the interview. Three trained interviewers visited cases and controls at home and also obtained information via a structured interview on participants’ medical history, lifetime smoking history (never, ex-smoker, current smoker), family history of bladder cancer, 20 year residential and lifetime occupational history [20].

Exposure assessment

The FFQ was based on a fixed response format consisting of nine categories for frequency of food consumption (never/seldom, 1–3 day/month, 1, 2, 3, 4, 5, 6, 7 days/week) and ten questions relating to food supplement intake (including vitamin A, vitamin C, vitamin D, vitamin E, multivitamins, other vitamins (open-ended question), iron, calcium, protein supplements, and an open-ended question to list other supplements). For some food items, alternative response options were provided, distinguishing between

five frequency categories ((almost) never, sometimes, 50% of the time, most of the time, (almost) always). Intake quantity was assessed based on standard household measures (5 categories: 1, 2, 3, 4, ≥ 5 table spoons/glasses/slices/portions, etc. or, alternatively, 1–2, 3–4, 5–6, 7–8, ≥ 9 tablespoons, etc.) and an open-ended response option (number of grams/pieces/plates, etc.). Each FFQ was electronically scanned and linked by an adapted version of the Nutritional Analysis of FFQ (NAF) computer software program developed by Instituto Nazionale Tumori (Epidemiology Unit) in Milan to the combined food composition table. This allowed for the conversion of household units into grams/micrograms etc. and the calculation of average daily intakes of food/groups and 29 nutrients.

Statistical analysis

Mineral and vitamin intake from both food sources and food supplements was calculated. Minerals included sodium, potassium, magnesium, phosphorus, copper, zinc, and iron. These and vitamins thiamin (B1) and riboflavin (B2) were all measured in milligrams (mg). The only other micronutrient, Vitamin D, was measured in micrograms (μg). To enable analysis based on tertiles, cut-off points were determined according to distributions among controls. The effect of these minerals and vitamins on the risk of bladder cancer was estimated by calculating odds ratios (ORs) and 95% confidence intervals (CIs) using unconditional logistic regression. We compared the odds for tertile intake categories between cases and controls, with the lowest tertile of intake as the reference category. Adjustment was made for the following potential confounders: age, sex, smoking characteristics (status, number of cigarettes smoked per day and the number of years of smoking cigarettes), occupational exposure (for longest occupation performed) to polycyclic aromatic hydrocarbons (PAHs) and aromatic amines (never vs. ever), and energy intake (kcal/day). Analyses were repeated to account for other factors as well, including alcohol, water, coffee, and tea consumption, which might modulate the action of minerals and water-soluble vitamins. However, this extended adjustment did not appreciably change the results.

Likelihood ratio tests were conducted to test for trend by assigning an integer value to each tertile of nutrient intake, e.g., 1–3, followed by entering the term as a continuous variable in the model. Additional analyses were performed to investigate the joint effects of micronutrients known for their biological interaction as well as the joint effects of minerals and established risk factors for bladder cancer: age, sex, and smoking characteristics (status, number of cigarettes smoked per day and number of years of smoking cigarettes). For this purpose, micronutrient intake level, age, cigarettes per day, and duration of smoking cigarettes

were cut into two categories based on either tertile scores (minerals; highest plus middle vs. lowest tertile) or median values (other variables; high vs. low) obtained from controls. Smoking status was dichotomized into current/non-current smoker, age into younger or older than 62 years, cigarettes smoked per day into less or more than 15 cigarettes per day, and duration of smoking into less than or more than 10 years of cigarette smoking. Study subjects were stratified into four categories according to intake level for each pair of minerals (high–high, high–low, low–high, and low–low). Subsequently, ORs were calculated comparing the odds for each category between cases and controls, with the low–low category as the reference category and using logistic regression analysis to adjust for potential confounding by age, gender, smoking characteristics, occupational exposure, and energy intake. Interaction between micronutrients (highest versus lowest intakes) and age, sex and smoking characteristics was tested by comparing the log likelihood of regression models with and without the relevant interaction term. We estimated the false-positive report probability (FPRP), expressing the probability of a spurious association between nutrient intake and bladder cancer risk, even in the case of a statistically significant finding. This procedure takes into account three factors that may influence the probability of a false-positive finding: (1) prior probability of a true association, (2) alpha level, and (3) statistical power to detect an odds ratio for the alternative hypothesis at a given p value or alpha level [27]. We used previously reported arbitrary values for this test: 0.25 (prior probability for an OR of 1.5), 0.5 (upper limit of noteworthiness), and 0.05 (alpha level) [27, 28]. Additionally, observed P values were calibrated and used to transform a preselected prior probability of a true association (10%) to the lower bound of the posterior probability of no association (null hypothesis) [29]. The Stata 10 statistical software program [30] was used for all analyses except for calibration of p values (Excel). Two-tailed p values < 0.05 were considered to reflect statistical significance.

Results

A total of 586 participants (200 cases and 386 controls) were included in the study. Data from 11 participants (2 cases and 9 controls) reporting extreme energy intake, that is < 1 st or > 99 th percentiles were excluded from analyses. Therefore, dietary data from a total of 198 cases and 377 controls were included in the analyses of this study.

Table 1 presents the study characteristics of the participants in the Belgian case–control study on bladder cancer. There were a greater percentage of males in both groups, and this percentage was higher in cases than controls

Table 1 Study characteristics of participants in the Belgian case–control study on bladder cancer

	Cases n (%)	Controls n (%)	p -Values
<i>Sex</i>			< 0.001
Women	27 [14]	151 [40]	
Men	171 (86)	226 (60)	
<i>Age</i>			< 0.001
Mean (SD)	67.6 (9.9)	64.2 (9.6)	
<i>Smoking status</i>			< 0.001
Non-current	32 (16)	156 (41)	
Current	166 (84)	221 (59)	
<i>Smoking duration</i>			< 0.001
< 10 years	33 (17)	182 (48)	
> 10 years	165 (83)	194 (52)	
<i>Cigarettes/day</i>			< 0.001
< 15 /day	86 (43)	259 (69)	
> 15 /day	112 (57)	117 (31)	
<i>Occupational exposure^a</i>			0.11
No	169 (85)	338 (90)	
Yes	29 [15]	38 [10]	
<i>Micronutrient Intake</i>	Cases mean (SD)	Controls mean (SD)	p -values
Sodium (mg)	4,096.08 (2269.37)	4,600.64 (4706.48)	0.08
Potassium (mg)	4,991.91 (1766.33)	5,296.59 (2565.05)	0.10
Calcium (mg)	1,127.16 (570.99)	1,194.46 (840.17)	0.26
Magnesium (mg)	436.46 (165.29)	461.99 (227.31)	0.12
Phosphorus (mg)	1,898.29 (769.42)	1,940.38 (841.74)	0.56
Zinc (mg)	14.47 (6.35)	14.73 (7.45)	0.66
Copper (mg)	2.53 (1.72)	2.78 (2.00)	0.12
Iron (mg)	21.28 (8.76)	23.11 (18.11)	0.10
Vitamin D (μ g)	7.24 (17.92)	8.54 (18.22)	0.41
Thiamin (mg)	1.67 (0.77)	1.77 (0.91)	0.17
Riboflavin (mg)	1.92 (0.78)	2.03 (1.01)	0.15

n number, SD standard deviation, mg milligrams, μ g micrograms

^a Occupation exposure never vs. ever PAHs and aromatic amines

(86 and 60%, respectively). On average, cases were older than controls ($p < 0.001$). Furthermore, cases were more often current smokers, smoked more cigarettes per day, and had smoked for a longer period than controls. There were no statistically significant differences between cases and controls for the average daily intake of the micronutrients under investigation (Table 1). Similarly, exposure to occupational carcinogens and intake of major dietary factors: energy (kcal), fat, water, alcohol, and tea and coffee were not statistically significantly different between cases and controls (data not shown).

The ORs and 95% CIs for dietary intake of minerals and vitamins and bladder cancer are presented in Table 2. A positive association was observed between intake of

Table 2 Adjusted odds ratios (OR) and 95% confidence intervals (CI) for tertile categories of mineral and vitamin intake and bladder cancer status

	Tertile of daily intake	1st (lowest)	2nd	3rd (highest)	<i>p</i> -trend
Adjusted for age, sex, smoking status, number of cigarettes smoked per day, number of years of cigarette smoking and exposure to occupational carcinogens (exposure to PAHs and aromatic amines) and energy intake (kcal/day)	<i>Sodium (mg/day)</i>	1313.7–3164.5	3164.6–4248.2	≥4248.3	
	Case/control	64/125	70/126	62/125	
	OR (95% CI)	1.0 (reference)	1.36 (0.82–2.24)	1.37 (0.74–2.55)	0.29
	<i>Potassium (mg/day)</i>	1872.3–4218.2	4218.3–5533.5	≥5533.6	
	Case/control	70/124	64/127	62/125	
	OR (95% CI)	1.0 (reference)	0.80 (0.49–1.31)	1.02 (0.57–1.85)	0.99
	<i>Calcium (mg/day)</i>	349.9–873.5	873.6–1252.1	≥1252.2	
	Case/Control	65/125	71/127	59/125	
	OR (95% CI)	1.0 (reference)	1.32 (0.81–2.15)	1.77 (1.00–3.15)	0.049
	<i>Magnesium (mg/day)</i>	125.3–370.4	370.5–482.5	≥482.6	
	Case/control	74/125	63/127	60/125	
	OR (95% CI)	1.0 (reference)	0.99 (0.60–1.63)	1.00 (0.54–1.83)	0.99
	<i>Phosphorus (mg/day)</i>	799.8–1542.9	1543.0–2087.1	≥2087.2	
	Case/control	57/125	72/126	65/125	
	OR (95% CI)	1.0 (reference)	1.73 (1.03–2.92)	1.82 (0.95–3.49)	0.06
	<i>Zinc (mg/day)</i>	4.7–11.1	11.2–15.5	≥15.6	
	Case/control	61/125	69/125	68/126	
	OR (95% CI)	1.0 (reference)	1.53 (0.91–2.55)	1.55 (0.83–2.91)	0.16
	<i>Copper (mg/day)</i>	0.6–1.8	1.9–2.7	≥2.8	
	Case/control	84/125	61/125	52/126	
	OR (95% CI)	1.0 (reference)	0.81 (0.50–1.32)	0.68 (0.38–1.21)	0.19
	<i>Iron (mg/day)</i>	6.5–16.9	17.0–22.7	≥22.8	
	Case/control	60/125	60/126	77/126	
	OR (95% CI)*	1.0 (reference)	1.01 (0.60–1.69)	1.64 (0.89–3.01)	0.11
	<i>Vitamin D (μg/day)</i>	0.2–2.0	2.1–3.7	≥3.8	
	Case/control	68/125	58/125	72/126	
	OR (95% CI)	1.0 (reference)	0.76 (0.47–1.24)	1.03 (0.62–1.72)	0.93
	<i>Thiamin (mg/day)</i>	0.7–1.3	1.4–1.7	≥1.8	
	<i>B1</i>				
	Case/control	63/124	71/127	58/125	
	OR (95% CI)	1.0 (reference)	1.18 (0.71–1.94)	1.07 (0.58–1.99)	0.79
	<i>Riboflavin (mg/day)</i>	0.7–1.5	1.6–2.1	≥2.2	
	<i>B2</i>				
	Case/control	66/125	73/127	57/125	
	OR (95% CI)	1.0 (reference)	1.56 (0.95–2.56)	1.35 (0.73–2.49)	0.28

calcium and bladder cancer (OR: 1.77; 95% CI: 1.00–3.15; *p*-trend = 0.049). Although there was an increased association of bladder cancer with a higher consumption of phosphorus, this was not statistically significant (OR: 1.82; 95% CI: 0.95–3.49; *p*-trend = 0.06). No associations were observed between any of the other minerals or vitamins and the risk of bladder cancer. These included: sodium, potassium, magnesium, copper, zinc, iron, vitamin D, thiamin, and riboflavin.

As higher odds of bladder cancer were observed for both calcium and phosphorus additional analyses were conducted to examine the joint effects of these minerals

together and with other key metabolically interacting micronutrients, magnesium and vitamin D. Table 3 shows the ORs and 95% CIs for all four inter-related micronutrients and bladder cancer. Participants who had the lowest intake of magnesium and were in the highest category for both calcium and phosphorus intake had a statistically significantly higher odds of bladder cancer (OR: 3.45; 95% CI: 1.48–8.04; OR: 3.91; 95% CI: 1.45–10.49, respectively). Levels of vitamin D had no effect on the association between calcium and bladder cancer, whereas lower levels of vitamin D in combination with a higher intake of phosphorus did significantly increase the odds of bladder

Table 3 Adjusted ORs and 95% CIs for minerals that biologically interact with each other and the risk of bladder cancer

Calcium </>873.6 mg	Phosphorus </>1,543 mg	Case	Control	OR (95% CI)
+	+	116	219	1.30 (0.64–2.63)
+	–	14	33	1.35 (0.58–3.18)
–	+	21	33	3.02 (1.01–9.02)
–	–	47	92	1.00 (reference)
Calcium </>873.6 mg	Magnesium </>370.5 mg			
+	+	101	212	1.24 (0.63–2.43)
+	–	29	40	3.45 (1.48–8.04) ^a
–	+	22	39	2.16 (0.75–6.21)
–	–	46	86	1.00 (reference)
Calcium </>873.6 mg	Vitamin D </>2.1 µg			
+	+	97	179	1.59 (0.89–2.84)
+	–	33	73	1.55 (0.66–3.65)
–	+	33	71	0.79 (0.38–1.65)
–	–	35	54	1.00 (reference)
Phosphorus </>1,543 mg	Magnesium </>370.5 mg			
+	+	117	228	3.26 (1.10–9.65)
+	–	20	24	3.91 (1.45–10.49) ^a
–	+	6	23	0.64 (0.20–1.99)
–	–	55	102	1.00 (reference)
Phosphorus </>1,543 mg	Vitamin D </>2.1 µg			
+	+	102	189	1.74 (0.91–3.33)
+	–	35	63	4.25 (1.44–12.55) ^a
–	+	28	61	0.98 (0.45–2.13)
–	–	33	64	1.00 (reference)

Adjusted for age, sex, smoking status, number of cigarettes smoked per day, number of years of cigarette smoking, exposure to occupational carcinogens (never vs. ever exposure to polycyclic aromatic hydrocarbons and aromatic amines), and energy intake (kcal/day)

^a Remained robust (i.e., statistically significant at an alpha = 0.05 with a prior probability of 0.25, for an odds ratio of 1.5 and cut-off level of 0.5 for false-positive report probability)

cancer (OR: 4.25; 95% CI: 1.44–12.55). A similar effect was observed for a higher intake of phosphorus combined with a lower intake of calcium (OR: 3.02; 95% CI: 1.01–9.02), and higher phosphorus combined with higher magnesium intake (OR: 3.26; 95% CI: 1.10–9.65). It should be noted, however, that none of the *p* values for interaction between any of the inter-related micronutrients were statistically significant (data not shown).

We performed FPRP calculations to further examine the probability of the statistically significant associations observed between bladder cancer and calcium intake overall and for the combinations of minerals and vitamins indicated in Table 3. Assuming a 25% prior probability of a true association (OR = 1.5) and applying the noteworthy level of 0.5 previously reported in other epidemiological studies [28, 31], we found noteworthy results for high calcium intake (false-discovery probability = 0.35), for the increased odds of bladder cancer resulting from low magnesium and corresponding high intakes of both calcium (false-discovery probability = 0.32) and phosphorus (false-discovery probability = 0.42) and high phosphorus with low vitamin D intake (false-discovery probability = 0.47). There was an excessive probability, however, the increased odds of bladder cancer for participants with the highest intakes of phosphorus and corresponding high intake of magnesium (0.55) and low intake of calcium (0.58) were false-positive findings. No associations were below the 0.5 criterion for noteworthiness when we repeated the FPRP estimations using a lower prior probability of 10%. Calibration of the statistically significant *p* values obtained for the positive association between calcium intake and bladder cancer risk and the higher odds associated with low magnesium intake and both higher calcium and phosphorus intakes and low vitamin D together with high phosphorus intake yielded minimum posterior probabilities of the null hypothesis of no effect (conditional error probabilities) of 0.79, 0.32, 0.46, and 0.50, respectively, based on a 10% prior probability of the alternative hypothesis. The minimum posterior probabilities for the same statistically significant findings were lower for a prior probability of 25% (data not shown).

No interaction was detected between calcium intake and sex and smoking characteristics (status, number of cigarettes smoked per day and number of years of cigarette smoking). However, we did observe an increased risk of bladder cancer associated with a higher intake of calcium among older participants compared with the younger age group (OR: 1.90; 95% CI: 1.08–3.36). Being male and belonging to the older age group also appeared to increase the risk of bladder cancer for participants with a higher intake of phosphorus (OR: 1.93; 95% CI: 1.08–3.44 and OR: 2.02; 95% CI: 1.05–3.92, respectively). The actual *p* values, however, for interaction between these micronutrients and age and sex were not statistically significant. Whereas all FPRP results were noteworthy using a prior probability of 0.25 only the potential joint effects of phosphorus and age remained noteworthy at the lower prior probability of 0.10 (data not shown). A statistically significant interaction was observed between vitamin D intake and the number of cigarettes smoked per day (*p*-interaction = 0.02), although the reduced odds of bladder cancer

for heavy smokers (>15 cigarettes/day) with the highest intake of vitamin D was not statistically significant (OR: 0.61; 95% CI: 0.33–1.12).

Discussion

Main findings

From our investigations into the effect of dietary minerals and vitamins on the risk of bladder cancer, we observed a statistically significant positive association with calcium intake. Although not statistically significant, we also detected an increased odds of bladder cancer associated with a higher intake of phosphorus. Further analyses revealed that participants with lower levels of magnesium and corresponding higher intakes of calcium and phosphorus had increased odds of bladder cancer. We also observed an increased risk of bladder cancer for older participants with higher intakes of both calcium and phosphorus. A higher intake of phosphorus also appeared to increase susceptibility of the disease for men and study subjects with low vitamin D intake. Furthermore, vitamin D intake appeared to interact with cigarette smoking resulting in a possible protective effect among the heaviest smokers. We did not detect any other potential associations, trends, or interactions.

Comparisons with existing evidence

According to the second expert report by the World Cancer Research Fund/American Institute for Cancer Research [32], the influence of calcium on cancer risk varies according to body site. The positive association between calcium and bladder cancer that we observed is in agreement with the results of a Spanish multi-centre case-control study [9], although in the Spanish study the effect disappeared after adjustment for saturated fat intake. However, our findings disagree with the results of three American studies, one large prospective cohort study [6] and two case-control studies [5, 7], and of a Dutch prospective cohort study [10] that reported no association with calcium intake.

A complete assessment of the effect of calcium intake cannot be obtained without taking its metabolic interactions with other food constituents such as phosphorus and vitamin D into account [15, 33]. For example, high intakes of phosphorus have been reported to be able to affect the calcium balance in the body [15]. As for the relationship with vitamin D, both calcium and phosphorus are considered to be the “main dietary regulators” of vitamin D levels in humans [33]. At increased concentrations,

calcium has been reported to reduce levels of vitamin D [34]. Alternatively, dietary phosphorus has the ability to influence vitamin D levels either way based on serum calcium and phosphorus concentrations [33]. Phosphorus is available from a wide range of dietary sources such as dairy products, meat, poultry, fish, and food additives [15]. Population differences in dietary sources, intake levels, and mineral balances may explain why an American study [7] reported a negative association and we conversely detected a positive association, both of borderline statistical significance, between dietary phosphorus and bladder cancer.

Measuring vitamin D exposure is difficult because it is available from two different sources: diet and sunlight (UV-B radiation) [35]. An ecological study [36] conducted in the US reported an inverse association between the solar source of vitamin D, UV-B exposure and bladder cancer. We only had data regarding dietary intake available and failed to identify an association between vitamin D intake overall and bladder cancer risk, which was in agreement with reports from a large US prospective study [6]. However, when we evaluated the modifying effects of inter-related micronutrients we found that participants with high phosphorus and low vitamin D intakes appeared to be at increased risk of bladder cancer. We also observed an interaction between the number of cigarettes smoked per day and vitamin D intake. Although not statistically significant, heavy smokers in the highest group for vitamin D intake had a reduced odds of bladder cancer. A possible association between smoking and vitamin D is consistent with findings from a Danish cross-sectional study of 510 perimenopausal women [37] that reported decreased serum vitamin D levels among smokers in the study (50% of participants).

The potentially protective effect of vitamin D has been attributed to its role in cell differentiation, proliferation, and apoptosis [38]. An American study [7] reported a possible reduced risk of bladder cancer among older participants with the highest intake of vitamin D. Many factors may influence the nutritional status such as vitamin D levels of older individuals. These include: living conditions (e.g., aged care facilities), comorbidities and the regular use of multiple medications and dietary supplements e.g., calcium [8].

Down regulation of the active form of vitamin D (1,25-dihydroxy vitamin D₃) due to high intakes of calcium and phosphorus may explain the increased risk of bladder cancer we observed in the older age group [33]. Similarly, a higher intake of phosphorus may have upset the balance with vitamin D and contributed to the increased odds of bladder cancer for the men in our study. A cross-sectional study [39] investigated the relationship between vitamin D and other minerals, specifically calcium and phosphorus, on bone mass in perimenopausal women. This study [39]

reported a negative association between the dietary calcium to phosphorus ratio and serum levels of vitamin D.

Magnesium is another mineral that metabolically interacts with vitamin D, calcium, and phosphorus [39]. In agreement with the few previous studies that investigated this mineral [6, 7], we did not detect any association between bladder cancer and magnesium as such. However, low levels of magnesium in conjunction with elevated levels of calcium and phosphorus appeared to exacerbate the risk of bladder cancer. It has been previously reported that high intakes of calcium may lead to magnesium deficiency [40]. As a mineral involved in more than 300 enzymatic reactions within the body, magnesium is clearly an important micronutrient. It should be noted, however, that in excessively high doses it can be toxic to certain individuals such as those with advanced renal disease [41].

Other minerals and vitamins have been previously investigated in this study population [42]. In an earlier study [42], no statistically significant associations were observed between bladder cancer incidence and intake of vitamin C, retinol equivalents or vitamin E after adjustment for age, sex, smoking characteristics, and occupational exposure to carcinogens. However, a statistically significant negative association was reported between serum levels of selenium and bladder cancer risk [42].

Currently, few epidemiological studies have investigated the effects of the remaining minerals and vitamins for which we found no association: sodium [5–7], potassium [6, 7], zinc [17, 18], iron [6, 7, 9], thiamin [5–7], riboflavin [5–7, 19], and copper. Only three of these studies [7, 18, 19] disagreed with our findings for these micronutrients and reported inverse associations between bladder cancer and intake of zinc (borderline statistically significant for total zinc intake) [18], thiamin (older individuals) [7], and riboflavin [19]. To our knowledge, this is the only epidemiological study that has investigated the association between copper intake and bladder cancer risk. Considering the current lack of information and the fact that many of these minerals and vitamins share common food sources and may biologically interact mutually and with other nutrients further investigation of these micronutrients is warranted [43].

Strengths and limitations of our study

A major strength of our study is the detailed and validated FFQ [22, 23] used to collect dietary data from our study population [21]. Although dietary data were collected at only one point in time (previous 12 month period), the FFQ has been reported to be an adequate instrument for measuring usual (average) food and nutrient intake for the purpose of our study [21, 22]. While it is impossible to discount the possibility that diet may have changed due to

disease status, another case–control study on bladder cancer [19] has reported that preclinical disease is unlikely to cause any major dietary changes. Moreover, given the general lack of understanding of the role of diet in bladder cancer etiology, both among experts and in the community, we are quite confident that only non-differential misclassification in dietary intake reporting by cases and controls may have influenced the study outcomes. This is further supported by the fact that there were no statistically significant differences between cases and controls for major dietary components, energy, and fat intake.

Risk of selection bias is a potential limitation associated with the case–control design, however, as previously reported [44] the study participants were demographically fair representatives of the general population. Although there were a higher proportion of high risk groups of bladder cancer among the cases compared with the controls. These included older participants, men, and heavy smokers who were also the groups of individuals with imbalances in dietary intake of calcium, phosphorus, and vitamin D.

The relatively small sample size is another potential limitation of this study. As a consequence of low statistical power, small effects of nutrient intakes as both main and joint effects may go undetected. Additionally, the lower proportion of low risk groups (e.g., younger participants, women and non-smokers) among the cases further limits the power of the study to show potential associations for these low risk groups.

Another potential weakness associated with this type of study design is the issue of multiple testing. We attempted to address this by performing additional analyses such as the FPRP test and calibration of *p* values to evaluate potential false-positive findings [27, 29]. Of these two methods, only the calibrated *p* values provided some evidence, although weak, for the modifying effects of inter-related micronutrients on bladder cancer risk at the lower prior probability of 10%. While these calculations can provide additional support for potential effects they are limited by the difficulty in determining an appropriate prior probability [27]. Cross-validation with other studies was another means by which we attempted to assess statistically significant findings obtained from multiple investigations.

Conclusion

We found a positive association between calcium intake and bladder cancer. There was some evidence of an interaction between intakes of magnesium and both phosphorus and calcium and phosphorus and vitamin D intake. Sub-optimal levels and balances between the related micronutrients calcium, phosphorus, magnesium and

vitamin D may influence bladder cancer risk. Individuals most affected by these micronutrient imbalances appeared to be those generally considered being at highest risk of bladder cancer: older participants, males, and heavy smokers.

Our results need to be confirmed by other studies, and further investigation is clearly required to determine optimal intakes and balances between these and other inter-related nutrients.

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Conflict of interest The authors declare no conflict of interest.

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